Plant Physiol. (1981) 67, 115–119
0032-0889/81/67/0115/05/$00.50/0

Water Relations of Cotton Plants under Nitrogen Deficiency

III. STOMATAL CONDUCTANCE, PHOTOSYNTHESIS, AND ABSCISIC ACID ACCUMULATION DURING DROUGHT[1]

Received for publication April 8, 1980 and in revised form August 21, 1980

JOHN W. RADIN
Science and Education Administration, United States Department of Agriculture, Western Cotton Research Laboratory, Phoenix, Arizona 85040

ROBERT C. ACKERSON
Central Research and Development Department, Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898

ABSTRACT

Nitrogen nutrition exerted a strong effect on stomatal sensitivity to water stress in cotton. In well-watered plants grown with 0.31 millimolar N in the nutrient solution, stomata closed at a water potential of −9 bars even though the wilting point was below −15 bars. For each doubling of nutrient N level, the water potential for stomatal closure decreased by about 2 bars. Elevated intercellular CO_2 concentrations caused only slight stomatal closure regardless of N nutrition. Exogenous abscisic acid (ABA) greatly increased stomatal sensitivity to elevated CO_2 concentrations.

Plants subjected to water stress gave the following responses: (*a*) decreased stomatal conductance at ambient external CO_2 concentration; (*b*) increased stomatal sensitivity to elevated CO_2 concentrations; (*c*) decreased mesophyll conductance to CO_2; and (*d*) increased endogenous ABA content. All of these responses to stress occurred at a higher water potential in N-deficient plants than in normal plants. The results show that N nutrition and water stress interact to control ABA accumulation and the events regulated by that accumulation.

Stomatal movement and its regulation have been studied since the early days of plant physiology. It is now known that both irradiance and c_i[2] can strongly affect stomatal conductance (13). In addition, the phytohormone ABA causes stomatal closure, in part by sensitizing stomata to c_i (7, 20). The synthesis of ABA in leaves during water stress is believed to account for drought-induced stomatal closure (11).

Recently Radin and Parker (19) reported that stomata of N-deficient cotton plants were much more sensitive to water stress than those of normal plants. Stomata of N-deficient plants closed at ψs as high as −10 bars, whereas those of normal plants closed at about −20 bars. Stomatal closure in N-deficient plants was not a result of leaf senescence (17). The effects of N deficiency were consistent with the finding that N-deficient plants have increased levels of ABA (6, 10, 14). Because ABA increases stomatal sensitivity to c_i (7, 20), any effects of this endogenous ABA on stomata should be manifested as differences in the plots of stomatal conductance *versus* c_i. Here, we examine the effects of N nutrition and water stress on ABA accumulation, photosynthesis, and stomatal responses to c_i in cotton leaves.

MATERIALS AND METHODS

Plant Growth Conditions. Seeds of cotton (*Gossypium hirsutum* L. cv. Deltapine 61) were germinated and grown in a greenhouse in 14-liter pots containing a mixture of peat moss, vermiculite, and sand. The greenhouse was cooled by refrigeration, with temperatures ranging from about 35 C during early afternoon to 22 C at night. Peak PAR was generally 1,800 to 2,000 μE m^{-2} s^{-1}. After establishment, plants were thinned to two/pot. Three times weekly, enough nutrient solution was added to each pot to ensure substantial leaching. The nutrient solutions were a modified half-strength Hoagland solution containing up to 5 mM N as KNO_3 + $Ca(NO_3)_2$, but no reduced N. When N concentrations were less than 5 mM, K^+ and Ca^{2+} concentrations were maintained by substituting KCl + $CaCl_2$ for KNO_3 + $Ca(NO_3)_2$.

Imposition of Water Stress. Watering was discontinued after the fifth leaf above the cotyledons had fully expanded. Stomatal and photosynthetic parameters were followed in the fifth leaf as stress progressed. For diffusive resistance measurements in the greenhouse, ψ was determined in early afternoon with a pressure chamber. For gas-exchange work, ψ was determined (on a leaf other than the fifth) at approximately 9:00 AM, just before the plants were transferred to the growth chamber (see below). The progression of stress was considerably faster in high-N plants than in N-deficient plants because of treatment effects on both leaf area and stomatal behavior. Typically, plants grown on 5 mM N showed incipient afternoon wilting 4 to 7 days after watering. Plants grown on 0.62 mM N reached the same point in twice that time.

Treatment with ABA. A leaf at the fifth node on a well-watered plant was sprayed to runoff with a solution of 0.1 mM (±)-ABA (Sigma)[3] containing 0.1% (v/v) Tween 20 or Triton X-100. Gas-exchange characteristics were determined the following day. Leaves of high-N plants received three sprays. Leaves of N-deficient plants were generally more sensitive to ABA and received only one spray.

Diffusive Resistances. As drying progressed in the greenhouse,

[1] This is contribution No. 2770 of the Central Research and Development Department, E. I. du Pont de Nemours and Co.

[2] Abbreviations and symbols: c_i, intercellular CO_2 concentration; g_s, stomatal conductance to water vapor; g_m', mesophyll conductance to CO_2; ψ, water potential.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

diffusive resistances to water vapor transport were followed with a transit-time diffusion porometer as described earlier (19). All measurements were in the early afternoon, at the time of minimum ψ. Total leaf resistance was calculated from the parallel resistances of abaxial and adaxial surfaces. Immediately following this measurement, the leaf was excised and ψ was determined in a pressure chamber.

These results are presented as resistances rather than conductances because the method of calibration and use of the porometer produced considerable scatter at high conductances. At conductances less than the maximum (the most useful range of the porometer), treatment effects were most easily visualized in terms of resistances.

Gas Exchange in Controlled CO_2. Plants were placed in a controlled environment chamber to provide constant temperature (27 C) and ambient lighting (250 μE m^{-2} s^{-1} PAR). A small clip-on cuvette was attached to the appropriate leaf, enclosing a circular area of 5.9 cm^2. The cuvette was constructed of acrylic plastic and had provisions for dividing an airstream equally between abaxial and adaxial chambers. Normal airflow was 605 ml/min. Reflective film was placed on the face of the cuvette with a cutout for the assimilation chamber to prevent excessive heating of the leaf.

The airstream was made up by mixing commercial compressed air with N_2 containing 3% CO_2 (v/v) in a gas-mixing manifold. Flows from each cylinder, and the flow rate of the mixture, were monitored with float-type glass flowmeters. The compressed air contained very little CO_2 and no detectable moisture.

Leaf temperature was monitored with a 0.1-mm (4-mil) copper-constantan thermocouple junction (Omega Engineering, Stamford, CT) appressed to the underside of the leaf inside the cuvette and connected to a Wescor TH-65 thermocouple thermometer (Wescor Instruments, Logan, UT). Because the air was dry, transpiration in the cuvette was great enough to keep leaf temperatures at or below 30 C, except at very low stomatal conductances.

The photosynthetic light source was a 500-w tungsten halogen lamp with a fan for forced-air cooling, a focusing lens to provide high irradiance over a small area, and a built-in heat filter. This system was commercially available as a Kodak Carousel slide projector. PAR was measured with a Li-Cor LI-185 meter equipped with an LI-190S quantum sensor (Lambda Instruments, Lincoln, NE). The cuvette was positioned in the light beam to receive 2000 μE m^{-2} s^{-1}. This level saturated both stomatal opening and photosynthesis under all conditions.

Transpiration and Photosynthesis. The effluent airstreams from each side of the leaf were combined and passed into a psychrometer calibrated at the appropriate flow rate and temperature. Wet- and dry-bulb thermocouple temperatures were monitored with the Wescor thermocouple thermometer. Whenever the composition of the incoming mixture was altered, the stomata were allowed to equilibrate for at least 30 min before measurements were recorded. Transpiration rates were calculated from the steady-state increases in humidity across the leaf. Conductances were calculated by assuming (*a*) that the air in the substomatal cavity was saturated with water vapor at the temperature of the leaf and (*b*) that the effective humidity gradient was the arithmetic mean of the initial and final gradients. With the transpiration rates actually achieved, this second assumption led to errors of 5% or less when compared to Gaastra's (9) equations. Reported conductances are the unseparated stomatal + boundary layer conductances. Boundary-layer conductance, determined from evaporation from wet blotting paper, was about four times greater than the maximum leaf conductance.

Photosynthetic rates were followed by a modification of the technique of Clegg *et al.* (4). Three 10-ml gas samples were slowly withdrawn from the effluent airstream, and then three samples were taken from the incoming airstream. All samples were stored until analysis in disposable syringes in which the plungers had rubber tips for a tight seal. The needles were sealed to the barrels with cyanoacrylate adhesive, and the needle tips were inserted into rubber stoppers to prevent leakage. Gas samples did not change composition even when stored several hours in this fashion. Samples were injected into an N_2 stream that was passed through a Beckman 315A IR gas analyzer equipped with a Perkin-Elmer M-2 integrator set to operate in the peak height mode. Peak heights were converted to CO_2 concentrations from a standard curve. Photosynthetic rates were calculated from the differences in CO_2 concentrations.

The c_i was calculated according to Farquhar *et al.* (8).

g_m' values were determined from the slopes of curves relating photosynthetic rate to c_i (15). This method prevented errors resulting from changes in compensation point. However, because all measurements were made in air of normal O_2 content, photorespiration could still have affected the results.

Endogenous ABA Levels. The ABA levels of plants were followed for 7 days after discontinuance of watering. Discs 18 mm in diameter were cut from the fifth leaf of eight high-N and eight low-N plants in the early afternoon, and each group was combined into two replicate samples. The discs were immediately frozen on dry ice and stored at −40 C until lyophilization. ψs were measured on separate plants in the same pots.

ABA was extracted and determined as described earlier (1). Results are expressed as ng ABA/cm^2. Compared to concentrations on a dry weight basis, the units chosen tend to underestimate the ABA levels of low-N leaves relative to high-N leaves, because low-N leaves contain less dry weight per unit area, and they undergo less shrinkage during stress (18).

RESULTS

Diffusive Resistance in Normal Air. Greenhouse-grown plants showed stomatal responses to ψ in normal air (about 350 μl/l) which clearly depended upon N nutrition. With 0.31 mM N in the nutrient solution, resistance increased rapidly at a ψ of −8 bars and reached 10 s/cm at −9 bars (Fig. 1). As available N was increased to 5 mM (four successive doublings), the ψ for a resistance of 10 s/cm was decreased to −18 bars. These results are similar to data reported earlier (19). For further study of stomatal regulation, plants were grown at only two N levels: 5 mM (high-N) and 0.62 mM (low-N). These two treatments afforded the best combinations of growth rate, water use, leaf size, and stomatal differences for

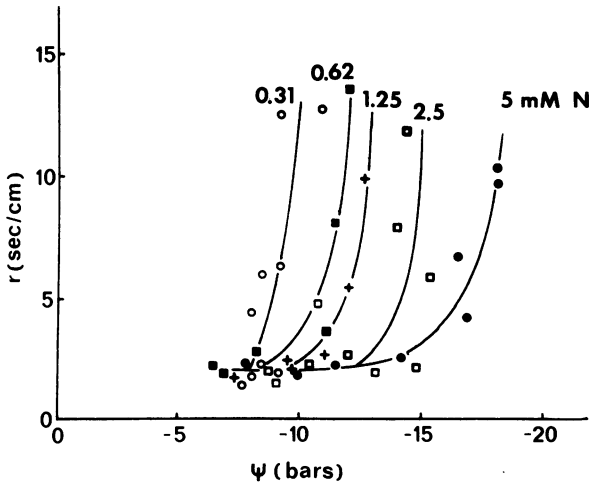

FIG. 1. Diffusive resistances for water vapor transport in leaves of plants grown at five levels of N nutrition. Plants were grown until the fifth leaf was fully expanded, at which time watering was discontinued. All measurements were on the fifth leaf.

convenient experimentation.

Responses to Increasing CO_2. g_s in well-watered plants declined, but only slightly, in response to increasing c_i (Fig. 2). There was little or no difference between high-N and low-N leaves in the response to increasing c_i. In contrast, leaves sprayed with 0.1 mM ABA responded very strongly to c_i. In such leaves, g_s decreased from about 0.2 to less than 0.05 mol m^{-2} s^{-1} when c_i was increased from 100 to 300 μl/l (Fig. 2). The effect of exogenous ABA was independent of N nutrition, although the low N stomata seemed to respond to lesser quantities of applied ABA (data not shown).

Photosynthetic rate was strongly affected by c_i and by N nutrition (Fig. 3). Typically, high-N leaves had a slightly lower compensation point, a slightly greater c_i for saturation, and a greater maximum rate of CO_2 uptake than low-N leaves. At saturating c_i, photosynthesis in the high-N leaves was about 50% greater than in the low-N leaves, reaching rates of almost 40 μmol m^{-2} s^{-1} (about 60 mg CO_2 dm^{-2} h^{-1}). Although treatment with ABA greatly modified stomatal behavior, it had less effect upon the other aspects of photosynthesis. In high-N leaves, ABA did not influence CO_2-limited photosynthesis at all ($c_i \leq 200$ μl/l) but, in low-N leaves, the g_m' at limiting CO_2 ($c_i \leq 175$ μl/l) apparently decreased by a small amount (Fig. 3). The loss of photosynthetic capacity in low-N leaves continued slowly for several days (data not shown) and presumably was an indirect effect of ABA rather than a direct effect (21).

Responses to Water Stress. The relationship between g_s and c_i changed as ψ declined; also, the effect of water stress upon this relationship depended upon N status. In high-N leaves, increasing c_i had little effect upon g_s until ψ had declined to -15.8 bars (Fig. 4, bottom). Although g_s decreased somewhat in these leaves in response to stress, the response was almost independent of c_i at -12.2 bars, and was only partially dependent upon c_i at -15.8 bars. In contrast, any decline in ψ of low-N leaves caused an immediate sensitization to c_i (Fig. 4, top). In these plants, most or all of the stomatal response to stress appeared to result from this induction of sensitivity to CO_2.

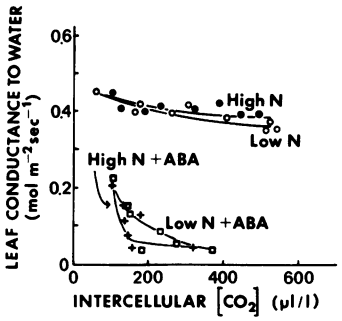

FIG. 2. Stomatal responses to c_i. High-N and low-N plants were grown on 5 and 0.62 mM N, respectively. ABA-treated leaves were sprayed with 0.1 mM ABA.

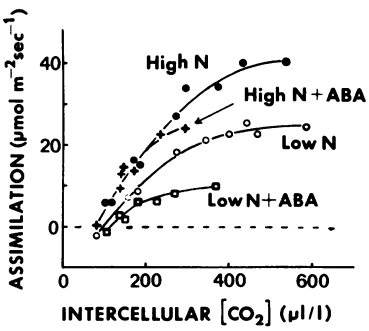

FIG. 3. Photosynthetic responses to c_i. Treatments were as described under Fig. 2.

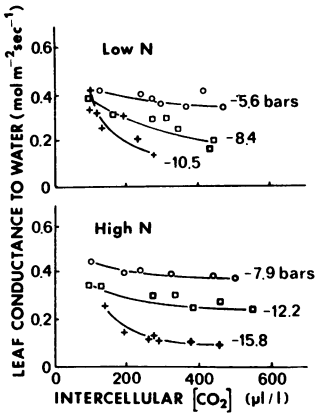

FIG. 4. Stomatal responses to c_i at various ψs during drying. Bottom, high N; top, low N.

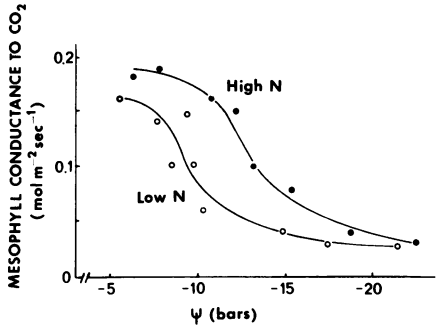

FIG. 5. g_m' values of high-N and low-N plants during drying. Conductances were determined from plots of photosynthetic rate versus c_i at limiting CO_2.

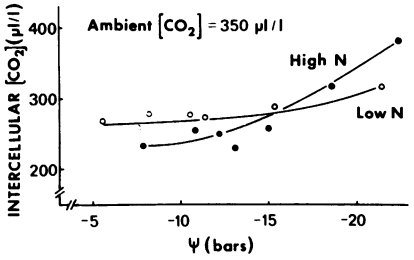

FIG. 6. c_i values of high-N and low-N leaves during drying. These concentrations were calculated from photosynthetic rates and g_s values at 350 μl/l ambient CO_2 concentration.

When photosynthesis is plotted against c_i, the slope of the curve at limiting CO_2 is proportional to g_m'. Before the onset of stress, low-N plants had a g_m' (slope) about 20% less than that of high-N plants (Fig. 3). (In terms of resistances, these values were 2.5 and 2.1 s/cm for low- and high-N leaves, respectively). As ψ declined, g_m' also decreased in both high-N and low-N leaves. Again, the effect of water stress on this relationship depended upon N status. In high-N leaves, g_m' declined very slowly until ψ reached -12 bars, at which point it began to drop rapidly (Fig. 5). Conductance continued to decrease with declining ψ until it reached a very small value. In low-N leaves, g_m' began to drop at about -7 bars and had decreased 50% at -10 bars (Fig. 5). Because of these changes in photosynthetic capacity, c_i tended to increase as ψ decreased, especially in the high-N leaves (Fig. 6). The c_i was slightly higher in low-N leaves above -15 bars, but this relationship was reversed below -15 bars (Fig. 6).

The increased sensitivity of stomata to c_i during stress (Fig. 4) presumably was tied to ABA accumulation in the leaf, because

exogenous ABA had a similar effect (Fig. 2). Analyses of leaf tissue confirmed that ABA levels increased in both high-N and low-N plants as ψ decreased, and the increases occurred at higher ψ in low N plants (Fig. 7). At any comparable ψ, ABA levels were greater in the low-N leaves than in the high-N leaves. During the 7 days without water, ψ decreased only to −17 bars in the low-N plants but reached −26 bars in the high-N plants. Despite this difference in ψ, the two groups of plants reached similar maximum ABA levels (Fig. 7).

DISCUSSION

Our results document a strong interaction of N nutrition and water stress on the physiology of the cotton leaf. In N-deficient leaves, both g_s and g_m' were much more sensitive to declining ψ than in normal leaves (Figs. 1, 4, and 5). The differences in stomatal behavior could not be explained by any effects of N deficiency on endogenous prestress ABA contents because N deficiency by itself did not cause stomata to imitate behavior seen in ABA-sprayed leaves (Fig. 2). Although differences in photosynthesis at high ψ (Fig. 3) caused a slightly greater steady-state c_i in low-N leaves than in high-N leaves (Fig. 6), the effect of that increased c_i was extremely small and, again, could not account for the differences in stomatal behavior. Further work showed that the differences in stomatal behavior arose only after the onset of water stress (Fig. 4). The induction of stomatal sensitivity to c_i by stress strongly resembled the effect of applied ABA (Fig. 2) and was correlated with increases in endogenous ABA during stress (Fig. 7). This correlation is weakened by the absence of ABA determinations at ψ values above −10 bars, when stomatal differences were evident at about −8 bars (Figs. 4 and 7). Part of this discrepancy resulted from sampling times: samples for ABA analysis were taken in early afternoon at the time of minimum ψ, but samples in the gas-exchange experiments were necessarily taken in the morning. Taken as a whole, the data provide strong evidence that ABA regulates stress-induced stomatal closure in both high-N and low-N plants.

From the ABA-like responses to stress (Fig. 4), it seems likely that low-N leaves began to accumulate ABA at a ψ as high as −8.4 bars. Earlier work (18) established that the wilting point of low-N plants was below −15 bars. Thus, the threshold turgor for ABA accumulation was apparently well above zero. In high-N leaves, ABA-like responses appeared at a ψ much closer to the reported wilting point (18). In this respect, the behavior of high-N plants was consistent with the observations of Pierce and Raschke (16), who reported a threshold turgor of zero for ABA synthesis in several plants including cotton.

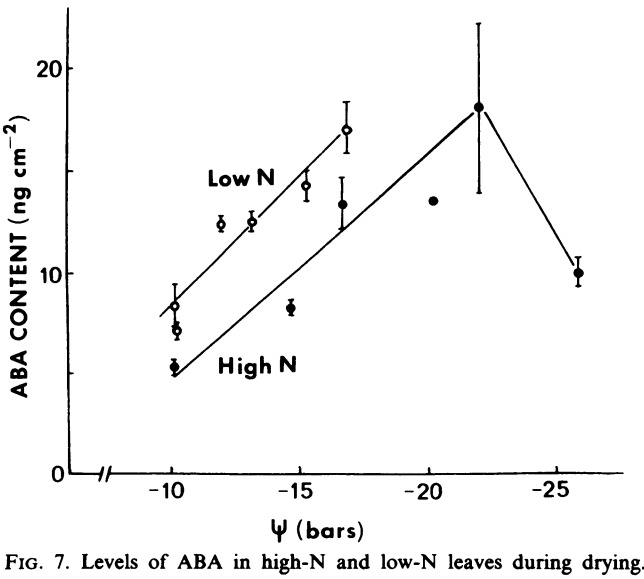

FIG. 7. Levels of ABA in high-N and low-N leaves during drying. Samples were all taken in early afternoon, at time of minimum ψ. Values shown are ± SE.

It has frequently been reported that N deficiency causes increased ABA levels in plants (6, 10, 14). Because N deficiency raises the threshold ψ for ABA accumulation, normal diurnal changes in ψ could initiate ABA accumulation, even in well-watered plants that do not approach wilting. This mechanism could produce elevated ABA levels independently of, or instead of, any direct effects of N deficiency on ABA metabolism. It seems likely that stomatal closure in N-deficient plants was regulated by ABA synthesized during stress (Fig. 4), but a possible role for ABA synthesized as a direct result of N deficiency remains unclear.

In both high-N and low-N leaves, the g_m' declined under water stress more or less in concert with the decline in g_s and increase in ABA content. Because of this decrease in photosynthetic capacity, c_i increased slowly in both treatments as the stomata closed (Fig. 6). These results differ widely from some previous work with cotton (22, 23; see also Hsiao (11) for discussion). A recent field study of cotton (2) also showed that water stress caused substantial nonstomatal inhibition of photosynthesis. The key to these findings may be the relatively slow development of stress. Similarly, osmotic adjustment in sorghum in response to stress occurred primarily under conditions of slow drying (12).

Boyer (3) has described the extensive work documenting nonstomatal inhibition of photosynthesis by water stress. Collatz (5), working with jojoba, also reported stress-induced decreases in g_m' which paralleled stomatal closure and stabilized c_i. The jojoba responses closely resembled those reported here and may be related to the concept that g_s and g_m' remain "balanced" (24). In cotton, nonstomatal inhibition of photosynthesis was unrelated to senescence, which occurred at much lower ψ (17). No further breakdown on the g_m' into its photochemical, biochemical, and transport components is possible from these gas-exchange experiments.

Acknowledgments—We thank J. S. Boyer, G. Guinn, and J. R. Mauney for helpful discussions and advice. L. L. Parker provided excellent technical assistance, and D. Brummett helped with equipment construction and maintenance.

LITERATURE CITED

1. ACKERSON RC 1980 Stomatal response of cotton to water stress and abscisic acid as affected by water stress history. Plant Physiol 65: 455–459
2. ACKERSON RC, DR KRIEG, CL HARING, N CHANG 1977 Effects of plant water status on stomatal activity, photosynthesis, and nitrate reductase activity of field grown cotton. Crop Sci 17: 81–84
3. BOYER JS 1976 Photosynthesis at low water potentials. Phil Trans R Soc Lond B 273: 501–512
4. CLEGG MD, CY SULLIVAN, JD EASTIN 1978 A sensitive technique for the rapid measurement of carbon dioxide concentrations. Plant Physiol 62: 924–926
5. COLLATZ GJ 1977 Influence of certain environmental factors on photosynthesis and photorespiration in *Simmondsia chinensis*. Planta 134: 127–132
6. DAIE J, SD SEELEY, WF CAMPBELL 1979 Nitrogen deficiency influence on abscisic acid in tomato. Hortscience 14: 261–262
7. DUBBE DR, GD FARQUHAR, K RASCHKE 1978 Effect of abscisic acid on the gain of the feedback loop involving carbon dioxide and stomata. Plant Physiol 62: 413–417
8. FARQUHAR GD, DR DUBBE, K RASCHKE 1978 Gain of the feedback loop involving carbon dioxide and stomata: theory and measurement. Plant Physiol 62: 406–412
9. GAASTRA P 1959 Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. Meded Landbouwhogesch Wageningen 59: 1–68
10. GOLDBACH E, H GOLDBACH, H WAGNER, G MICHAEL 1975 Influence of N deficiency on the abscisic acid content of sunflower plants. Physiol Plant 34: 138–140
11. HSIAO TC 1973 Plant responses to water stress. Annu Rev Plant Physiol 24: 519–570
12. JONES MM, NC TURNER 1978 Osmotic adjustment in leaves of sorghum in response to water deficits. Plant Physiol 61: 122–126
13. MEIDNER H, TA MANSFIELD 1968 Physiology of Stomata. McGraw-Hill, London
14. MIZRAHI Y, AE RICHMOND 1972 Abscisic acid in relation to mineral deprivation. Plant Physiol 50: 667–670

15. NOBEL PS, TL HARTSOCK 1978 Resistance analysis of nocturnal carbon dioxide uptake by a crassulacean acid metabolism succulent, *Agave deserti*. Plant Physiol 61: 510–514
16. PIERCE M, K RASCHKE 1980 Correlation between loss of turgor and accumulation of abscisic acid in detached leaves. Planta 148: 174–182
17. RADIN JW 1981 Water relations of cotton plants under nitrogen deficiency. IV. Leaf senescence during drought and its relation to stomatal closure. Physiol Plant 51: In press
18. RADIN JW, LL PARKER 1979 Water relations of cotton plants under nitrogen deficiency. I. Dependence upon leaf structure. Plant Physiol 64: 495–498
19. RADIN JW, LL PARKER 1979 Water relations of cotton plants under nitrogen deficiency. II. Environmental interactions on stomata. Plant Physiol 64: 499–501
20. RASCHKE K 1975 Simultaneous requirement of carbon dioxide and abscisic acid for stomatal closing in *Xanthium strumarium* L. Planta 125: 243–259
21. SHARKEY TD, K RASCHKE 1980 Effects of phaseic acid and dihydrophaseic acid on stomata and the photosynthetic apparatus. Plant Physiol 65: 291–297
22. TROUGHTON JH 1969 Plant water status and carbon dioxide exchange of cotton leaves. Aust J Biol Sci 22: 289–302
23. TROUGHTON JH, RO SLATYER 1969 Plant water status, leaf temperature, and the calculated mesophyll resistance to carbon dioxide of cotton leaves. Aust J Biol Sci 22: 815–827
24. WONG SC, IR COWAN, GD FARQUHAR 1979 Stomatal conductance correlates with photosynthetic capacity. Nature 282: 424–426